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QUANTIFYING HUMAN VITAMIN KINETICS WITH AMS

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INTRODUCTION

Tracing vitamin kinetics at physiologic concentrations has been hampered by a lack of quantitative sensitivity for chemically equivalent tracers that could be used safely in healthy people. Instead, elderly or ill volunteers were sought for studies involving pharmacologic doses with radioisotopic labels. These studies fail to be relevant in two ways: vitamins are inherently micronutrients, whose biochemical paths are saturated and distorted by pharmacological doses; and while vitamins remain important for health in the elderly or ill, their greatest effects may be in preventing slow and cumulative diseases by proper consumption throughout youth and adulthood. Neither the target dose nor the target population are available for nutrient metabolic studies through decay counting of radioisotopes at high levels. Stable isotopic labels are quantified by isotope ratio mass spectrometry at levels that trace physiologic vitamin doses, but the natural background of stable isotopes severely limits the time span over which the tracer is distinguishable. Indeed, study periods seldom ranged over a single biological mean life of the labeled nutrients, failing to provide data on the important final elimination phase of the compound. Kinetic data for the absorption phase is similarly rare in micronutrient research because the phase is rapid, requiring many consecutive plasma samples for accurate representation. However, repeated blood samples of sufficient volume for precise stable or radio-isotope quantitations consume an indefensible amount of the volunteer's blood over a short period. Thus, vitamin pharmacokinetics in humans has often relied on compartmental modeling based upon assumptions and tested only for the short period of maximal blood circulation, a period that poorly reflects absorption or final elimination kinetics except for the most simple models.

AMS TRACING IN HUMANS

We apply the high sensitivity of accelerator mass spectrometry (AMS) to solve these difficulties by tracing ^{14}C-labeled micronutrients in very small blood samples over long periods of time after a single physiologically relevant dose. Even for a nutrient with a mean biological life of one month, 100 nanoCuries (133 kBq) of ^{14}C imparts a lifetime added radiation exposure to the volunteer equivalent to that obtained in 15 minutes of a high altitude plane flight. A 70 kg person naturally contains a similar amount of ^{14}C to the experimental dose. The radiation exposure in AMS-based nutrient studies becomes of little or no concern when compared to the commonly accepted risk of a normal activity. Similarly, the sensitivity of AMS reduces sample size needs to only tens of microliters of blood, volumes that permit frequent sampling and high density data sets that are able to constrain and guide proper compartmental modeling of nutrient distribution and fate.

We have experience with kinetic studies of 4 micronutrients in healthy populations of young people (median age ≈ 25 years): folate, beta carotene, lutein, and tocopherol. The hydrophilic nutrient, folate, had a surprisingly wide range of bioavailabilities (65 - 95%) quantified by mass balance of the tracer, yet showed similar kinetics among all volunteers (7 women, 6 men; median age of 24), as seen in Figure 1. A large enterohepatic recycling of folate equal to 5 times the ingested dose was discovered through compartmental modeling of the high density data [1].

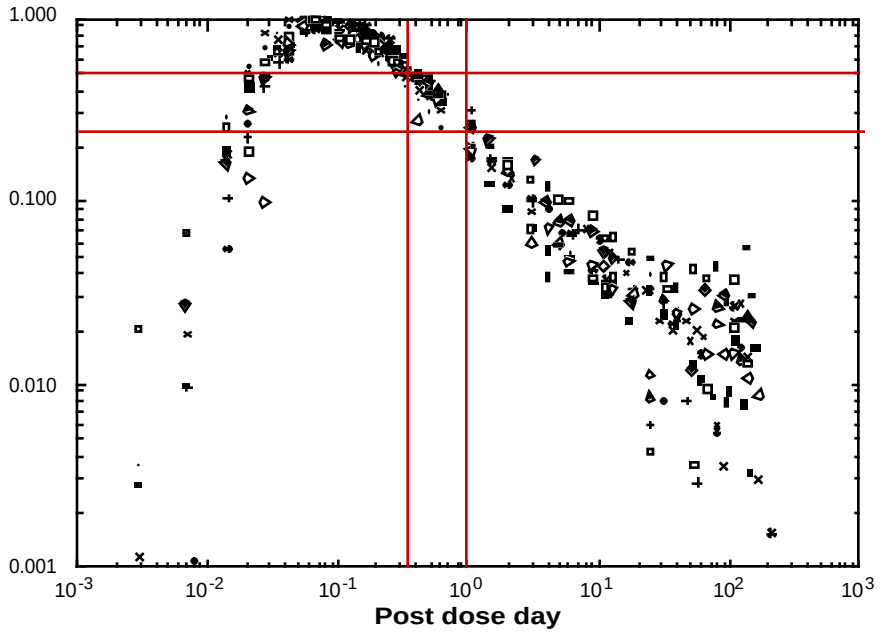

Figure 1. Plasma kinetic profiles of [^{14}C]-folate on log-log scales for 13 human volunteers spanning 10 minutes to 1 week after the 35 µg, 100 nCi dose. Profiles are normalized to 1 at the maximum plasma concentrations of each. Primary elimination half life was 16 hours.

The lipophilic nutrients carotene, lutein, and tocopherol revealed complex and extended kinetic profiles that are not easily modeled [2,3]. Beta-carotene absorption and metabolism was studied in two volunteers after 1 mg, 200 nCi doses with and without 10,000 IU supplements of vitamin A. Plasma samples were fractionated by HPLC to obtain the kinetics of metabolic products from the [^{14}C]-beta-carotene. Figure 2 shows the concentrations of carotene, retinyl esters, and retinol in plasma samples as functions of time from 10 minutes to 45 days post dose. Despite the low need to produce retinol from the ingested carotene, the vitamin A supplemented period used carotene as a retinal source at 81% the level of the unsupplemented period. Vitamin A supplementation also increased the intestinal metabolism and absorption of carotene into circulating retinyl and retinol pools [4].

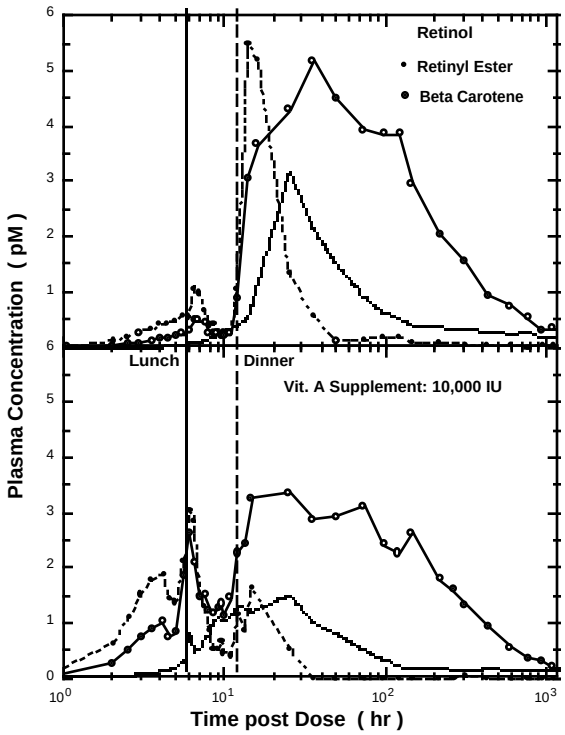

Figure 2. Plasma concentrations of beta-carotene and metabolites as functions of time after a single 1 mg dose of isotope-labeled compound. The lower pane shows the plasma responses due to a similar dose with vitamin A supplementation having started 2 weeks prior to dosing and continuing 2 weeks post dose.

We hypothesize that the large change in intestinal response to the carotene dose is due to improved epithelial health in the intestine from the added vitamin A. The supplement did not change the conversion level of carotene to retinol after the main absorption event at 10 hours. The integrated retinol from 10 to 1200 hours is 26% of the integrated carotene concentration with only a 0.7% drop seen with supplementation.

PHYSIOLOGY FROM PHARMACOKINETICS

The quantitative sensitivity of AMS allows high sample density after physiologic doses of vitamins and other nutrients using only microliter samples of human plasma for kinetic and metabolic determinations. The high data density reveals physiologic information above and beyond the simple pharmacokinetic data. Models of folate metabolism and circulation are highly constrained by the large data sets from many people, permitting discovery of an unexpected large enterohepatic circulation that returns demethylated folate to the liver for remethylation. Kinetic profiles of beta-carotene metabolism show an unexpected enhancement of metabolism and intestinal absorption due to vitamin A supplementation. AMS is, thus, not only a tool for pharmacokinetics, but also a technology that strengthens the value of pharmacokinetic data as probes of physiology and physiological change.

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